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Original Research

Rib fractures: Could we accelerate the healing?

Rib fractures healing

Mustafa Calik[1], Saniye Goknil Calik[2], Mustafa Dagli[3], Mustafa Cihat Avunduk[4]
[1]Department of Thoracic Surgery, Health Sciences University, Konya Training and Research Hospital,
[2]Department of Emergency and First Aid Program, Vocational School of Health Services KTO Karatay University,
[3]Department of Cardiovascular Surgery, Health Sciences University, Konya Training and Research Hospital,
[4]Department of Pathology, Necmettin Erbakan University Meram Faculty of Medicine, Konya Turkey

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Abstract

Aim: We thought about "How fractures are improving more rapidly." For this purpose, we made an experimental model to investigate the effect of zinc supplementation on the development of new bone in young rabbits. Material and Method: Six weeks of age, male New Zealand white rabbits were evaluated in four groups, each including five subjects. To simulate the RFs, subperichondrial costal cartilage resections beginning from the third costal cartilage were carried out in the right hemithorax according to groups. Rabbits in Group 1 and 2 underwent partial resection of the two ribs; rabbits in Group 3 and 4 underwent total resection of those. Zinc was administered by intraperitoneal injection of 6 mg/kg/ day for four weeks after the surgery for group 2 and 4. The animals were followed-up at the twenty-fourth week of their life. Results: We analyzed histologic changes in the bone. There were statistically significant differences for osteoblasts and osteoclasts among all subgroups. Histologic consolidation was significantly increased by zinc supplementation. According to the literature, in our study, while zinc stimulates osteoblastic bone formation, suppresses osteoclastic bone resorption. Discussion: Although the proper treatment of rib fracture (RF), long-term disability and persistent chest wall pain frequently develop and may take several months to recover, leads to the high hospital, medicine, labor, and social burden. Our findings indicate that zinc supplementation accelerates the consolidation of ribs. Zinc can be used to increase the bone maturation such as the site of new bone formation in RF.

Keywords

Zinc; Supplementation; Rib Fracture; Rabbits

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Corresponding Author: Mustafa Calık, Department of Thoracic Surgery, Health Sciences University, Konya Training, and Research Hospital, 42090, Meram, Konya, Turkey.
T.: +90 3323236709 F.: +90 3323236723 E-Mail: drmcalik@hotmail.com
ORCID ID: https://orcid.org/0000-0001-9963-5724

Introduction

Mortality due to trauma ranks third after cardiovascular diseases and cancers among the causes of adult death worldwide [1]. Rib fractures (RFs) are a common injury affecting greater than 145,000 people each year in the United States, with at least one-third of patients requiring hospital admission [2]. It is clear that this number is higher, considering that 77% of patients with rib fractures are not applying to emergency departments.

Historically RF's treatment is symptomatic, based on pain control with analgesics including narcotics and non-steroidal anti-inflammatory drugs (NSAIDs), respiratory physiotherapy, and specific treatment of associated complications [3]. Approximately 31% of them will encounter complications including nosocomial pneumonia, prolonged respiratory failure, prolonged hospitalization, or death, due to coexisting injuries, age, and comorbidities [2-4].

Hydroxyapatite crystals, which are the mineral structure of bone, contain many trace elements such as zinc (Zn), first described in 1941. Although these trace elements are a very small part of the bone mass, they are very important and vital for bone metabolism. [5,6]. Zinc promotes bone formation in both people and animals by stimulating osteoblast proliferation and differentiation, increases stimulating osteoblastic bone mineralization by way of alkaline phosphatase activity and collagen synthesis. Also, while inhibiting bone resorption suppresses differentiation of osteoclasts [6-8]. Despite up-to-date treatment, long-term disability and persistent chest wall pain frequently develop and may take several months to recover, leads to the high hospital, medicine, labor and social burden [2-4]. We thought about "How fractures are improving more rapidly." For this purpose, we made an experimental model to investigate the effect of zinc supplementation on the development of new bone in young rabbits.

Material and Methods

This experimental study was carried out at the Experimental Medicine and Research Centre of local University. A total of the same generation six weeks of age, male New Zealand white rabbits (740-980 gr) were randomly evaluated in four groups, each including five subjects. All animals received humane care and were used in compliance with standards established by the European Convention for Animal Care and Use of Laboratory Animals. The rabbits were fed a standard pelleted diet and were allowed to access tap water *ad libitum.* The animals were housed in standard individual cages on a 12-h light/dark cycle at room temperature in a humidity-controlled environment. The local Animal Ethical Committee approved all study-related procedures (2006/03). This study was approved and funded by the School of Medicine Animal Care and Investigational Committee in our institution. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Study Design

Groups

The subjects were divided into four equal groups, each containing five rabbits. Before the operation, the rabbits were numbered and weights were noted in grams. The entire process was performed under general anesthesia under sterile conditions. Subperichondrial costal cartilage resections beginning from the third costal cartilage were carried out in the right hemithorax. Rabbits in Group 1 and 2 underwent partial resection of the two ribs; rabbits in Group 3 and 4 underwent total resection of those. Costochondral joints were saved in partial resections and removed in total resections. Three-quarters of the costal cartilages were removed in partial resections.

Anesthesia

Ketamine HCl induced general anesthesia (Ketanest, Pfizer Pharma GmbH, Karlsruhe, Germany) 15-20 mg/kg i.v. or 20-25 mg/kg i.m. moreover, maintained by Xylazine (Alfazyne 2%; Alfasan International. BV, Woerden, Netherlands) 0.5-1 mg/kg i.v. or 1-2 mg/kg i.m. If needed, same dosages of Ketamine HCl and Xylazine were repeated with reflex responses (pedal reflex, palpebral, and corneal reflexes) to keep the anesthesia depth constant. Body temperature was monitored by inserting a heat probe into the ECG and rectum with the aid of needle electrodes. Heating lamps were used to keep the animals at 37±5°C body temperature during the surgical preparation and working period. The mean anesthesia time was 12-15 min for each rabbit. No animals were treated with any local and systemic antibiotics.

Operation technique

Subjects were placed in the right lateral decubitus position on the operating table with spontaneously breathing.Then, chests were shaved and cleaned with a povidone-iodine solution (10% povidone-iodine, Betadine, Kansuk, Istanbul, Turkey); lateral thoracotomy incision was carried out after infiltrating with 1% lidocaine and with 1:100000 epinephrine (Jetokain Simplex ampule; Adeka Pharmaceutical Company, Istanbul, Turkey). The pectoral muscles were divided along the laterally to allow exposure of the costal cartilages and thoracic wall. A horizontal incision was made over the third and fourth costal cartilage level. Subperichondrial costal cartilage resections were carried out in the right hemithorax according to groups. In each subject, bleeding and pneumothorax control was performed with sterile serum saline. After all controls, the chest wall was closed with continuous sutures by anatomical layers without any grafting.

Postoperative Care and Follow-Up

Pain control in animals was provided by Tradomol HCl (Contramal, 100 mg 2 ml, Abdi Ibrahim Ltd., Istanbul, Turkey) 1-2 mg/kg/day i.m. for five days during the postoperative period. The animals were followed-up untill the rabbits were accepted to go into adulthood at the twenty-fourth week of their life. All animals were euthanized with a lethal IV dose of Non-barbiturate anesthetic (Ketamine/Xylazine) painlessly according to the existing instructions established by the latest report of the AVMA Panel on Euthanasia. Three times the anesthetic dose was used for euthanasia [9].

Zinc protocol

Zinc sulfate ($ZnSO_4$) (Merck, CAS number 7446-20-0 Darmstadt, Germany) was in crystalline form. The solution was prepared by dissolving 1 mg/ml in distilled water to make the stock solution. It was stored at 4°C at which it could remain stable for at least a month. The solutions to be injected were prepared by diluting stock solutions using 0.9% NaCl (0.1 mg/1 ml) on a daily basis. Working solutions were used within 1 hour following the preparation. Zinc was administered with 0.5 ml of saline by intraperitoneal injection of 6 mg/kg/ day at 10-12 every day for four weeks after the surgery for Groups 2 and 4 [10].

Pathological Evaluation

All materials were decalcified in 10% buffered formaldehyde for 48 hours after the fixation period until they were tempered enough to be cut with a microtome. Tissue specimens from appropriate sites were then taken for the autotechnical follow-up, embedded in paraffin and stained with Hematoxylin & Eosin (H&E) and sectioned with a microtome to calculate the osteoclast, osteoblast, lymphocyte count and the area of new bone formation (Figure 1). All stained preparations were examined with Nikon Eclipse E400 light microscope (Nikon Corporation. Minato-ku, Tokyo Japan). Care was taken to select as possible as the same areas for each case in evaluating. The selected areas were scanned with a Nikon Coolpix 5000 digital camera (Nikon Corporation. Minato-ku, Tokyo Japan) with a microscope mounted at the same microscope magnification. At the same time, Nikon Stage Micrometer (MBM11100, Nikon Corporation. Minato-ku, Tokyo Japan) images were also taken for calibration with the same microscope magnification. All images were transferred to a PC environment for analysis with Clemex Vision Lite 3.5 (Clemex Technologies inc. Longueuil, Quebec Canada) (Figure 2). First, the length was calibrated with Nikon Stage Micrometer (MBM11100, Nikon Corporation. Minato-ku, Tokyo Japan). After the calibration, the area to be examined was determined as 38732.7μm2. The osteoblasts, osteoclasts, and lymphocytes on the 38732.7 μm2 areas selected on the digital images of H&E stained preparations were marked and automatically counted by the mentioned image analysis program. The damaged cells were excluded from the evaluation during the examination.

Statistical analysis

All data were assessed using the Statistical Package for Social Sciences (SPSS) 18.0 portable for Windows (SPSS Inc, Chicago, Illinois, USA). In every subgroup, we analyzed histologic changes in the bone. The effect of zinc treatment between groups was evaluated by using the Kruskal-Wallis test and the Mann-Whitney U test. There were statistically significant differences for osteoblasts and osteoclasts between all subgroups (Table 1). A p-value (<0.05) was used to indicate a significant difference.

Results

Regardless of the cause of deaths animals were replaced with new ones. All rabbits have been included in our experiment lived up to the end of the study. There were no local complications such as skin reaction, wound infections, or bleeding around the operating. There were statistically significant differences for osteoblasts and osteoclasts among all subgroups (Table 1). Table 1 shows no statistically significant difference between groups in Groups 1 and 2 except fibroblasts, Lymphocytes, and VEGF when compared to in Zn (Groups 2 and 4) and non-Zn groups (Groups 1 and 3). The difference was in osteoblasts, osteoclasts, and fibroblasts between only Groups 1 and 2. Table 2 shows the mean and standard deviation values of all groups. While there was no difference in VEGF between the groups, the numerical difference was higher in Zn than in non-Zn groups. They were affected by the shape of the surgical dissection.

There was no difference in lymphocytes. However, there were more lymphocytes in the Zn group than in the non-Zn groups. Zinc application increased the number of lymphocytes. Besides, the number of lymphocytes was affected by the shape of the resected ribs.

There was no significant difference between the groups except for the Groups 1 and 2. There is only a significant difference between these groups. We believe that growth center conservation and limited resection are the causes of this difference. Although the same numbers of ribs were removed, there was no statistically significant difference between the Groups 3 and 4 in which the growth center was not preserved, and the mean and standard deviation values were higher. Fibroblasts increased in groups where the growth center was not preserved. There were more fibroblasts in the Zn group than non-Zn.

Osteoblasts were significantly higher in each group of zinc than in each group without zinc. However, the groups in which the growth center was resected (Groups 3 and 4) were lower than the non-resected groups (1 and 2). Even the positive effect of zinc did not prevent this decline. However, the partial resection in Group 1 was similar to the total resection in Group 4 with Zn. Zinc has the same effect on the conservation of the growth center.

Osteoclasts were statistically significant and low in Zn groups compared to those without zinc. However, in the groups in which the growth center was resected (Groups 3 and 4), it was more than the groups that were not resected (Groups 1 and 2). Zinc groups are less in zinc-free groups. Zinc decreases osteoclasts and increases bone mass. Osteoclasts are less in Zn groups than in non-Zn groups. It decreases osteoclasts, thus increasing bone mass and consolidation. Histologic consolidation was significantly increased by zinc supplementation. By the literature, in our study, while zinc stimulates osteoblastic bone formation, suppresses osteoclastic bone resorption.

Table 1. Comparative groups' t- and p-values

	Group 1-2		Group 3-4	
	t	P	t	P
VEGF (Vascular Endothelial Growth Factor)	-1,633	,141	-,232	,822
Lymphocyte	-1,386	,203	-1,535	,163
Fibroblasts	-6,693	,000	-,061	,953
Osteoblast	-4,788	,001	-5,200	,001
Osteoclast	3,674	,006	2,449	,040

Table 2. The mean and standard deviation of the four groups of the rabbits measured at 24 weeks of age (n = 5 for each group)

	Group 1	Group 2	Group 3	Group 4
VEGF (Vascular Endothelial Growth Factor)	4,8±1,48	6,0±0,70	6,0±1,22	6,2±1,48
Lymphocyte	6,4±2,96	8,6±1,94	10,6±3,20	13,6±2,96
Fibroblasts	5,8±1,30	11,4±1,34	14,2±6,53	14,4±3,36
Osteoblasts	18,8±2,28	25,4±2,07	13,6±1,34	18,8±1,78
Osteoclast	5,8±0,83	4,0±0,70	7,0±1,58	5,2±0,44

Discussion

Traffic accidents are the most frequent cause of hospitalization and RF, and anticipated to increase by 65% until 2033 in developing countries. For this reason, it seems certain that we will encounter more RF in the coming years. Chest pain depending on RFs is caused by broken bones and injured muscles. It is generally exacerbated by any movement of the chest wall including deep breathing and coughing in even normal breathing movements. The current standard treatments especially inpatients (patients be in the hospital) are based on powerful pain control intervention such as epidural analgesia, intrapleural local anesthetics instillation, rib blocks, and intravenous narcotics/NSAIDs. Outpatients with RFs are most commonly treated only with relatively powerless oral analgesics such as narcotics and NSAIDs (NSAIDs). After being discharged from the hospital these powerful treatments are terminated. Patients are to be left alone with severe pain that takes several weeks to heal [4]. Despite all treatment options, 31% of the cases get into complications such as nosocomial pneumonia, prolonged respiratory failure, long-term hospitalization, or death due to age, accompanying the comorbid disease, and traumas [2-4]. Although the number of RFs and the age of the patient are designated as the factors that increase mortality and morbidity in the literature, this situation is controversial [11]. While high morbidity is defined in patients older than 65 years, a similar high mortality and morbidity tendency has been described in younger patients [12]. The number of RF is similar. Whitson and colleagues showed that high mortality and morbidity were not associated with the number of RF in 35.467 disease series. However, they did not assess whether rib fractures worsened or exacerbated existing comorbidities or had any effect on the severity of the accompanying injuries [13]. On the other hand, Jones et al. identified five and more RFs as an independent cause of mortality in 98.836 series of disease. This needs to be explained [14]. Mortality that ranges from 2 to 20% and morbidity mostly due to the accompanying injuries are reduced with the control of pain in chest traumas and especially in RFs. They are reduced by the provision of pain and bone stability [15].

In the literature, there are a lot of experimental studies relating to Zn and bone healing. To our knowledge, this is the first study about the zinc and RF. It is the only trace element affecting the basic structure or functions of osteogenic enzymes involved in bone development and healing. [6]. In many parts of medicine including cardiothoracic surgery, oral surgery and orthopedics, controlled and guided bone growth is based as well on bone metabolism consisting of osteogenesis, bone modeling and bone remodeling [16]. According to the significant effect on the bone formation by zinc, we hypothesized that the in the treatment of rib fractures after median sternotomy the oral surgery can be used to accelerate healing. Our experimental model was similar to a rib fracture model, in addition, the growth center of the ribs was resected. [11]. In the literature, minimal cartilage resection and saving the growth center of the ribs at costochondral junctions has been suggested in order to produce faster healing [17]. Nevertheless, histologic consolidation was significantly increased by zinc supplementation even in the group where growth centers were resected (Table 2). In accordance with the literature, in our study, while zinc stimulates osteoblastic bone formation, it suppresses osteoclastic bone resorption.

Like in every study, there are some limitations in our study. Firstly, as an experimental animal study cannot entirely be applicable to humans, therefore, additional prospective human studies are needed. Secondly, the method of administration, dose, and additional cost of Zinc should be investigated in humans.

Conclusion

Although human beings have been trying to overcome chest trauma, especially rib fractures from the first people, even today, it is still painful and can be potentially disabling. The actual figures and costs are much higher than expected. Because of the inadequacy of existing or traditional treatment, 30% of patients do not return to entirely their work or normal activities. This results in social and economic costs both to the national healthcare system and to individuals in the form of lost productivity and decreased the quality of life. Any improvements to its treatment would have evenly had agreat benefit not only on the individual but also on society [4, 18]. Our findings indicate that zinc supplementation accelerates the consolidation of ribs. Zinc can be used to increase the bone maturation such as the site of new bone formation in rib fracture. Healing modulation or acceleration may alleviate pain, enhancing recovery and reduce disability on the treatment of RFs. Zinc supplementation could be a next step that we are looking for a long time.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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